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FOUR WATER-SOLUBLE MOUNTING MEDIA FOR MICROSLIDES

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ABSTRACT

Four mounting media for plant samples, particularly bryophytes, on microslides were surveyed: PVOH-glycerin, glycerin jelly, water-glass-glycerin, and pure glycerin with stabilized cover slips. Each was found valuable for particular uses. The new polyvinyl alcohol and glycerin mountant ("glycerine-glue") proved better than the others for general work. Details of formulae and technique are given.

There have been many publications recommending various water-soluble mounting media for making temporary or semi-permanent slide preparations for microscopic examination (e.g. Anderson 1954; Bowers 1964; Creaser & Clench 1923; Davis 1909; Frahm 1990; Lightowlers 1981; Sayre 1941, Zander 1983, 1997). Discussions by various bryologists on the bryological listserver Bryonet have been productive in describing details of methods of effectuating such mounts, with greater or lesser success.

Recent work in my lab has settled on four different mounting media, all based on glycerin (glycerol). Only glycerin has the several excellent qualities of being rather stable over time (low evaporation rate, high boiling point), being miscible with water, has least osmotic effect on cells, high index of refraction, speed of preparation, inexpensive, and, lastly but not least, being non-poisonous. Each of the four methods involves dealing with the fact that glycerin is a thick liquid and can slowly run off a slide that is tilted. Although keeping glycerin mounts in a sealed cabinet with a dish of glycerin to saturate the air may retard evaporation, glycerin will eventually evaporate and air will penetrate under the cover slip. All attempts to quickly seal (lute) a preparation so the glycerin *never* evaporates are here considered futile, as any glycerin or finger oil on the slide, or differential expansion of the glass and mountant, damages the seal. Glycerin can be attacked by microorganisms, so one can optionally add a crystal of thymol to avoid bacteria and fungi. Note that glycerin dissolves (eventually) calcium carbonate.

Much of the problem with glycerin-based and other mounting media, such as Hoyer's Solution (Anderson, 1954) or polyvinyl lactophenol (Frahm, 1990), is that osmotic effects may collapse the cells of the mounted specimen of sensitive, thin-walled species. Efforts to slowly infiltrate the specimen with glycerin, such as a drop on the margin of a water mount, are tedious and often unsatisfactory. The secret is to allow initial collapse when glycerin is applied to water-soaked material, but to then heat the slide at low temperature on a hot plate or coffee warmer. The cells of the material mounted in the water and glycerin mixture plump up quickly and stay that way as the water evaporates on the hot plate or later over time. If clearing is desired, first dip the moist plant in lactic acid for a minute or so (or heat in lactic acid) before preparing the mount. Do not stack slides for long as the pressure will squeeze glycerin from the mounts.

PVOH-GLYCERIN OR "GLYCERIN GLUE"

This is simply a 1 to 1 mixture of glycerin and a thick, syrupy solution of polyvinyl alcohol in water, or more conveniently, a ready-made polyvinyl alcohol-based glue, such as Elmer's Washable

Clear School Glue™ or Colorations™ Washable Clear Glue, both available on the Web. Add a small quantity of water to the pure glycerin first and stir in well to enhance later mixing with the glue. Heat will help disperse the glue in the slightly aqueated glycerin. The Elmer's and Colorations clear glues are apparently mostly or all polyvinyl alcohol in water. A test found that pure polyvinyl alcohol (polyvinyl alcohol 98.1–98.8%, Carolina Biological Supply) in powder form dissolved in water (about 20%, see Woods, 1997) to make a thick syrup acts much the same, but remained somewhat cloudy. Perhaps the commercial glues have a better mixing protocol. Premixed polyvinyl alcohol in water from Carolina Biological Supply worked as did the glues, demonstrating that PVOH was indeed the effective ingredient in the clear glues, but the four percent solution was too weak to set the glycerin well.

The index of refraction of PVOH-Glycerin remains high. When water in the glue evaporates in a day or two it makes a firm mount that is solid enough for mailing. If the cells of the mounted specimen collapse, heat on a hot plate or cup warmer, but collapse is rare.

Both glycerin and polyvinyl alcohol are slightly hygroscopic, but that seems to contribute no adverse effects. If PVOH powder is used rather than prepared glue, heat the powder in water vigorously for a long period to make sure the granules dissolve. Do not try to dissolve powdered PVOH in glycerin or glycerin-water as the solution becomes opaque.

Positive: No heating is necessary to melt the mountant. There are no restrictions on stains (its pH is neutral), and plants retain diagnostic colors with 2% KOH wetting solution. One can manipulate the specimen directly in the medium. The formula is simple and ingredients easily obtained. Old slides are easily soaked to remove the cover slips and refurbish. *Negative:* The exact ingredients in the clear glues are proprietary. Three or four drops of PVOH-Glycerine are necessary on a slide because the medium evaporates about 1/4 to 1/2 of its bulk in water. This is the best mounting medium I have ever used in more than 50 years of bryological study.

GLYCERIN JELLY

Take 1 packet (7 g) of Knox gelatin. Mix and let stand for a while in 50 ml cold water to hydrate. Heat but do not boil while still stirring, until the liquid is clear or at least there is no undissolved gelatin. Add glycerin to 300 ml. Heat gently for about an hour until the liquid is clear. Too much gelatin makes the jelly difficult to melt and included bubbles found on the slide will not burst.

Pour on a clean pan or plate with a flat bottom to make a thin layer. PVC (polyvinyl chloride) pans work well but any plastic that does not adhere to gelatin is acceptable. Leave uncovered (drape a cloth if air is dusty) overnight or a couple of days to allow most of the water to evaporate. Peel off the thin, flat sheet of glycerin jelly. Roll the sheet into a long tube. Slice the roll crosswise into neat, tight curlicues or helixes about 1/4 inch wide. Keep in a plastic box. Pinch off a small piece when wanted. The flat sheet of glycerin jelly will be hard to remove from the pan unless the water portion has evaporated. It is best to evaporate the water portion with heat since glycerin absorbs water, to some extent, from the air. If there are bubbles, reheat in a beaker (a water bath is helpful) and let stand as liquid.

Put plant material on a slide in water, or KOH solution (Zander 1993) or Aerosol solution (Zander 1997), soak well to eliminate air in cells. Take a strip of glycerin jelly and pinch off a small portion, put on the slide with the material, and heat on a hot plate or cup warmer (the kind that plugs in the wall is best). Try not to boil as bubbles are difficult to eliminate. Arrange material and gently add a cover slip. Although you can put a dropper bottle of glycerin jelly on a hot plate to keep it

liquid, the heat eventually turns the jelly brown, and the gelatin breaks down so that it will not harden. Some of the above is presented by Zander (1997).

Positive: Mounted plant material does not move at all; there is no sloshing or gradual settling to one side. The mount hardens in a minute and may be mailed when cool, almost immediately after labeling. Little water is present so no pockets develop from evaporation. *Negative:* Bubbles, if they form, seldom disappear. Heating must be carefully done. Operations on the material, e.g., sectioning, cannot be done in cool, solidified glycerin jelly.

WATER-GLASS-GLYCERIN

A water glass and glycerin mountant (WGG) was investigated. This seems to work well, but is not suitable for immediate mailing or other disturbance as it must dry for a day or so. The formula is simply 2 parts water-glass (sodium silicate solution 40–42 Baumé, Carolina Biological Supply), and 1 part glycerin that is previously mixed with a little water to help it dissolve in the water-glass.

Mix and stir well. Put in capped squeeze bottle or dropper bottle, but not a glass-stoppered bottle (the sodium silicate seals the plug). Several drops on a slide are necessary because much water will evaporate. The WGG solution will dry fastest around the cover slip edges and hold the slip on tightly in a day or two. The high index of refraction and tinting of leaf cells by the highly basic water-glass may allow easy anatomical analysis of *Sphagnum* species without staining. If a stain is needed, however, only basic stains are useful in this particular mounting medium. Safranin O, for instance, works fine, but toluidine blue, orcein, and methyl green precipitate out.

If the specimens have collapsed leaf cells after mounting, put the slide on a hot plate until the liquid under the cover slip just boils. Over a month or two of time, a whitish or brownish deposit may accumulate just within the margins of the cover slip, as water glass migrates to the edges and precipitates. This leaves a perfectly clear large central portion of the cover slip that is mainly glycerin held fast by the peripheral water-glass.

Creaser and Clench's (1923) paper advises far more water-glass (12:1 water glass to water) than is suggested above, but the medium is then too harsh osmotically. The present 2:1 method uses water-glass to hold the glycerin to the microscope slide, while Creaser and Clench's 12:1 method uses glycerin to keep the water-glass from crystallizing. Those who find that the WGG formula of 2:1 results in slides that are too syrupy might use 3:1.

Positive: No heating is necessary. The solution is basic and moss plants may show interesting, strong, characteristic and permanent color reactions similar to those obtained with KOH solution. *Negative:* The water-glass crystallizes out around the cover slip margins and looks messy. The large amount of water in the water-glass solution allows air pockets to form under the cover slip in thick mounts as it evaporates, and additional solution must be added. One is limited to basic stains.

PURE GLYCERIN WITH COVER SLIP STABILIZER

Soak plant in water or 2% KOH solution. Add 3-4 or more drops of glycerin. Heat to drive off most or all of the water over a coffee warmer. Put a clear PVOH glue as noted above around the cover slip margins using a small brush or applicator. The clear glue is used to hold the cover slip in place, not to prevent the glycerin from evaporating.

Because the glycerin mountant and PVOH stabilizer are both soluble in water, when the glycerin does begin to dry up, the slide is easily soaked or steamed to remove the cover slip, and the specimen remounted. One can, in fact, dunk the whole slide or that portion with the cover slip into diluted (half water and half glue) commercial clear glue, and let it dry on a slanted wire rack. The

glue covers the cover slip in a thin, flat, optically transparent film, and does not restrict casual examination.

Positive: The index of refraction is very high and cell details are superb. Few or no bubbles are evident after heating, while glycerin jelly often seems to retain any bubbles from heating. The mounts do not shrink because any water is already evaporated on heating. *Negative:* Do not leave anything heavy on the slide since it is easily disturbed. A tilted slide may allow fragments under the cover slip to migrate.

SUMMARY

Different methods are best for different applications. Glycerin jelly is best for making mounts for illustration as the plant parts do not migrate, and it is a proven long-term though not permanent mountant. Water-glass-glycerin is acceptable when color reactions of species to alkalis are studied. PVOH-glycerin is best for all-around quick mounts and is here recommended for general use. Pure glycerin has the highest index of refraction and thus provides the best viewing and may be optimal for critical or irreplaceable material, because old material, when glycerin is mostly evaporated, is easily retrieved with hot water or steam.

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